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TRITIUM RETENTION BY COWS AND STEERS AND TRANSFER TO MILK

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16 ABSTRACT

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Additional study of the milk to assess tritium incorporation in the various fractions showed half-times of 2.91 ± 0.14 and 43.7 ± 4.28 days for milk serum; 2.08 ± 0.56 and >50 days for milk protein; and 3.20 ± 0.15 and 60.7 ± 43 days for butterfat.

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TRITIUM RETENTION BY COWS AND STEERS AND TRANSFER TO MILK

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FOREWORD

Protection of the environment requires effective regulatory actions which are based on sound technical and scientific information. This information must include the quantitative description and linking of pollutant sources, transport mechanisms, interactions, and resulting effects on man and his environment. Because of the complexities involved, assessment of specified pollutants in the environment requires a total systems approach which transcends the media of air, water, and land. The Environmental Monitoring and Support Laboratory-Las Vegas contributes to the formation and enhancement of a sound integrated monitoring data base through multidisciplinary, multimedia programs designed to:

- develop and optimize systems and strategies for monitoring pollutants and their impact on the environment

- demonstrate new monitoring systems and technologies by applying them to fulfill special monitoring needs of the Agency's operating programs

This report presents the results of an investigation designed to evaluate the short- and long-term behavior of tritium in beef animals and dairy cows with emphasis on the resultant hazard to humans consuming meat and dairy products. It is hoped that this information will be of use to those individuals who must assess hazards from accidental release of tritium, designers of nuclear waste processing facilities and those responsible for nuclear reactor site selection. Additional information regarding this study may be obtained by contacting the Exposure Dose Assessment Branch of the Monitoring Systems Research and Development Division.

George W. Morgan
Director
Environmental Monitoring and Support Laboratory
Las Vegas

ABSTRACT

Eight lactating dairy cows and three steers received a single oral administration of tritiated water. Milk and blood from the lactating cows and blood from the steers were periodically collected and analysed for tritium content.

The tritium content of whole milk decreased with time giving a curve expressed as a three component exponential which yielded half-times of 1.04 ± 0.09, 11.1 ± 2.58, and >120 days. Tritium in the blood serum of steers decreased with half-lives of 4.05 ± 0.21 and 40.4 ± 9.82 days.

Additional study of the milk to assess tritium incorporation in the various fractions showed half-times of 2.93 ± 0.14 and 41.7 ± 4.28 days for milk serum; 2.08 ± 0.56 and >50 days for milk protein; and 1.28 ± 0.35 and 60.7 ± 41 days for butterfat.

CONTENTS

	Page
FOREWORD	iii
ABSTRACT	iv
LIST OF FIGURES	vi
LIST OF TABLES	vii
ACKNOWLEDGMENTS	viii
INTRODUCTION	1
CONCLUSIONS	2
RECOMMENDATIONS	2
METHODS	3
RESULTS	4
DISCUSSION	14
REFERENCES	15

LIST OF FIGURES

Number		Page
1.	Average concentration of tritium in whole milk following a single oral administration of 100 mCi of tritiated water to four dairy cows.	6
2.	Average concentration of tritium in milk components following a single oral administration of 200 mCi of tritiated water to four dairy cows.	9
3.	Average concentration of tritium in blood serum following a single oral administration of tritiated water to dairy cows and beef animals.	11
4.	Average concentration of tritium in blood serum following a single oral administration of 65 mCi of tritiated water to three beef cattle.	13

LIST OF TABLES

Numbers		Page
1.	Tritium Concentration in uCi/l of Water Or Its Equivalent in Whole Milk and Components Following A Single Oral Administration of 100 millicurie of Tritiated Water	5
2.	Tritium Concentration in uCi/l of Water Or Its Equivalent in Milk Components Following A Single Oral Administration of 200 millicurie of Tritiated Water	7
3.	Tritium Concentration in Blood Serum of Dairy Cows Following A Single Oral Administration of 100 millicurie of Tritiated Water	10
4.	Tritium Concentration in Blood Serum of Dairy Cows Following A Single Oral Administration of 200 millicurie of Tritiated Water	10
5.	Tritium Concentration in Blood Serum of Beef Cattle Following A Single Oral Administration of 65 millicurie of Tritiated Water	12

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INTRODUCTION

As an isotope of hydrogen, tritium is incorporated into essentially all components of biological systems. Tritium is produced as a result of nuclear fusion and, to a certain extent, nuclear fission explosives, including those used in the nuclear stimulation of natural gas formations (Moghissi and Carter, 1971). Tritium is also produced in all nuclear power reactors both as fission and activation products. Due to the presence of large quantities of tritium in the initial stages of operation, it is expected that tritium production in fusion reactors will be substantially higher than in fission reactors. Because of the problems associated with separation and disposal of tritium from nuclear waste, dilution is presently used as the disposal method for this radionuclide. However, it is expected that in the foreseeable future tritium will be separated and contained.

Cow's milk is recognized as one of the major routes of the intake of radioactive pollutants into the human body. Attempts to study tritium metabolism in bovines have been numerous. Black et al.,(1964) studied the turnover rate of tritium in a total of 17 lactating and nonlactating cattle. They observed no difference in the turnover rate of tritium in body water (T_{b}) in nonlactating as compared to lactating animals. Their T_{b} values ranged from 3.8 to 4.1 days with an average of 3.5 days. However, Leschbacher et al.,(1965) studied a number of physiological parameters in dairy cows using four lactating and two nonlactating animals. They observed T_{b} values for tritium of 1.0 days for lactating and 5.2 days for nonlactating cows.

The incorporation of tritium into the organic fraction of milk subsequent to the intake of tritiated food has also been repeatedly studied. Lubran and Corsini (1960) and Glascock and Wright (1962) studied tritium transfer into certain components of milk subsequent to the intake of tritiated triglycerides. These authors clearly showed the importance of considering the chemical form of tritium intake when determining the composition of tritiated compounds in milk. In a series of papers Kirchmann et al., studied tritium behavior in dairy cows (Kirchmann et al., 1969; Kirchmann et al., 1971; and Van den Hoek and Kirchmann, 1971). These authors observed values of 1 to 5 days for the T_{b} of tritium in lactating cows. They also observed an increase of tritium concentration in milk solids if tritiated grass was fed to the cows when compared to intake as tritiated water. Values for the biological half-life of tritium in milk solids were compared to those in body water.

Potter et al.,(1972) also studied tritium behavior using one dairy cow. They observed a two-component exponential excretion rate corresponding to half-lives of 3.1 and 40 days, respectively. They also showed a substantial incorporation of tritium into the organic component of the milk.

Due to concern over the continued release of large quantities of tritium

into the environment and because of the lack of consistant information on the behavior of tritium in components of man's food chain, this study was undertaken in dairy cows and beef animals to evaluate both the short- and long-term behavior in one of the more significant sources of exposure.

CONCLUSIONS

1. Little significant difference was noted in the rate of tritium in body water turnover in lactating dairy cows versus steers.

2. Although whole milk is the major route of exposure of humans consuming tritiated dairy products, additional information on the turnover rate of tritium in milk solids is necessary before an accurate determination of radiation dose can be made.

RECOMMENDATIONS

In the event of contamination of agricultural areas with tritium, the potential hazard to humans ingesting tritiated dairy products must be carefully evaluated. As more knowledge is gained of the metabolism of specific tritiated compounds, it may be found that incorporation of tritium into nucleic acid, via deoxyribonucleic acid (DNA), may influence all aspects of biological activity and have greater effect than previously ascribed. Further studies with a biological receptor are necessary to accurately estimate exposure/dose resulting from the consumption of tritium.

METHODS

These studies were carried out at the Nevada Test Site on the experimental farm operated by the U.S. Environmental Protection Agency for the U.S. Energy Research and Development Administration. The dairy animals selected for the study were either Holstein or Jersey cows 4 to 9 years of age, were in their 30th to 100th day of lactation, and were producing 20 to 31 kilograms (kg) of milk per day. The beef animals were 4- to 6-year-old Hereford steers. All the animals were allowed free access to water and alfalfa hay. In addition, the dairy cows received a pelleted commercial dairy feed containing 16% protein.

The cows were milked with a milking machine, and blood samples from the cows and steers were taken by jugular venipuncture. Tritiated water was administered to each animal following collection of milk and/or blood for background activity determinations. The tritiated water was placed in gelatin capsules containing starch and administered orally using a balling gun. Milk samples were collected twice daily from the dairy animals, and blood samples

were collected from all animals hourly for the first 12 hours and then at longer intervals during the remainder of the project. Milk samples were stored in 4-liter polyethylene containers to which 10 milliliters (ml) of formaldehyde was added to retard spoilage. Blood samples were centrifuged and the serum portion was removed for tritium analysis. All samples were processed for analysis within 24 hours.

This study was conducted as a series of three consecutive experiments. The milk transfer portion of the study utilized two groups of four lactating dairy cows (three Holsteins and one Jersey) in each group. Each of the cows was given a single 100-millicurie(mCi) dose of tritiated water. The milk from this group was analyzed for tritium activity in the whole milk.

The total activity in the milk was determined directly by liquid scintillation counting utilizing an internal standard. The scintillation liquid consisted of p-xylene containing 7 grams (g) of 2,5-diphenyloxazole (PPO) and 1.5 g of bis-(o-methylstyryl)benzene (bis-MSB) per liter mixed with Triton N101 (Rohm and Haas, Philadelphia, Pennsylvania) in a volume ratio of 2:1. This mixture could incorporate up to 10 ml of water or milk in a 25-ml vial (Lieberman and Moghissi, 1970). Counting efficiency was determined by using an internal standard with identical system properties (Moghissi and Carter, 1968). The whole milk was mixed thoroughly and two aliquots of each sample were prepared with one of them having a known quantity of tritium added.

The second group of dairy cows received 300 mCi of encapsulated tritiated water each. Milk from these cows was separated into milk, serum, butterfat, and protein fractions and the tritium activity of each fraction was determined.

Milk serum was separated by the addition of trichloroacetic acid (15 g/100 ml). The presence of about 100 milligrams(mg) of hydrogen ions per gram in the acid was regarded as acceptable and within expected errors. The mixture was allowed to stand for about 30 minutes and filtered, and the filtrate was distilled. The tritium activity in the distillate was determined by liquid scintillation counting.

Cream was separated from the milk by cryogenic centrifugation. Fat was separated from cream using heat and 100 ml of Triton and 25 grams of hexametaphosphate per liter of cream as described by Norowitz (1970). The purity of the fat was checked using the Babcock test (Hausler, 1972).

The proteins were separated from the milk serum and dried for several days in an oven at a temperature of 40° C. The dried protein was weighed and the organically bound tritium separated by combustion (oxidation) in a Parr bomb. After the water of combustion was collected, its tritium content was determined by liquid scintillation counting (Moghissi et al., 1975).

After installation of a large scale azeotropic distillation system (Moghissi et al., 1973), the protein and serum separation was repeated using this procedure and the results indicated a reasonable agreement between the two methods.

In order to determine the difference in blood tritium levels between

lactating and nonlactating animals, three Hereford steers were each administered 65 mCi of tritiated water in the same manner as the dairy cows. Blood was collected from all three groups of cows and the serum portion analyzed by direct liquid scintillation counting.

The data were analyzed by performing a nonlinear least squares regression on a sequence of successively more complex mathematical models in the class of sums of exponential terms functions. The best of the regression functions was chosen using the general linear hypothesis test and half-lives were calculated from the exponential coefficients of the best-fit regression.

RESULTS

The individual values for whole milk are shown in Table 1 for the four cows receiving 100 mCi of tritiated water. The half-lives for tritium transfer to whole milk are presented graphically in Figure 1. This figure represents the mean of the whole milk concentration values listed in Table 1. Tritium in the whole milk of cows appears to decrease with a 3.04 ± 0.09-day half-life during the 20 days following oral administration of 100 mCi of tritiated water. The transfer of tritium to milk then changed to exhibit a slope with a half-time of 11.1 ± 2.58 days followed by a much longer half-time of >120 days.

In order to determine the biological half-time of tritium in the different milk components, a second experiment was conducted in which each of the dairy cows received 200 mCi of tritiated water. The results of this experiment are shown in Table 2 and Figure 2. The initial half-times shown by the milk components indicate little differences between the milk serum and the butterfat with half-times of 2.93 ± 0.14 days and 3.28 ± 0.35 days, respectively. The protein fraction of the milk exhibited a considerably shorter half-time of 2.08 ± 0.56 days. The longer half-time portion of the curves showed the normal variance of results with time and lower activity. The shortest half-time was indicated by the tritium activity in the serum portion of the milk. This was found to be 41.7 ± 4.28 days. The results of the protein analysis showed that a relatively constant incorporation of tritium into protein occurred during the 2nd and 3rd months after administration, followed by a half-time of >50 days for the remainder of the study. The incorporation of tritium into butterfat decreased with a longer half-time of 60.7 ± 41.0 days and then increased during the latter part of the study.

The blood levels of tritium were similar for both the lactating dairy cows and the beef animals during the first 12 hours after administration as shown in Tables 3 and 4 and Figure 3. The tritium levels in blood serum from beef cattle shown in Table 5 were averaged and the half-lives determined to be 4.05 ± 0.21 days and 40.36 ± 9.82 days as shown in Figure 4.

[illegible]

[illegible]

[illegible]

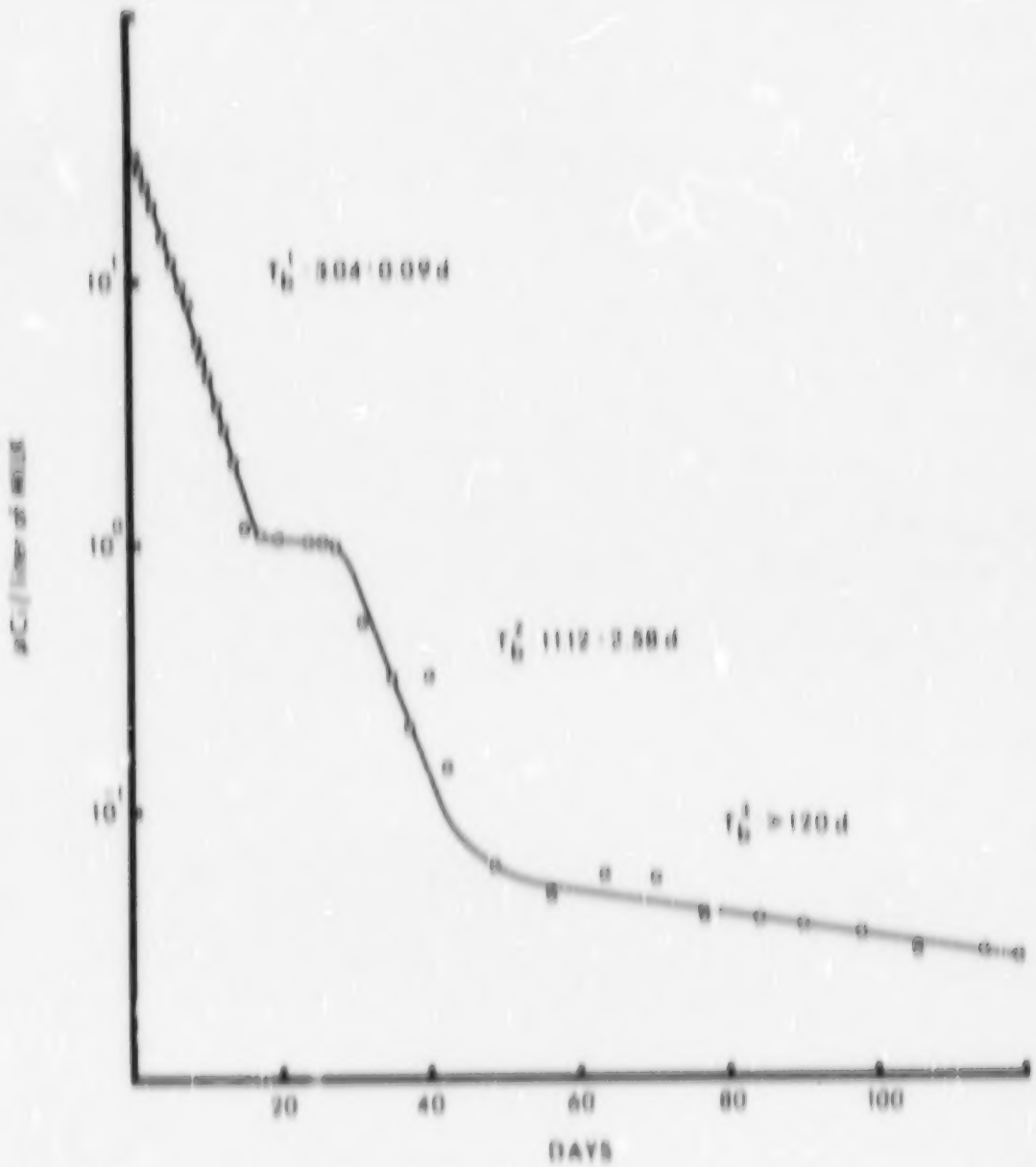

Figure 1. Average concentration of tritium in whole milk following a single oral administration of 100 mCi of tritiated water to four dairy cows.

TABLE 1. [illegible] CONCENTRATION IN μCi/l OF WATER OR ITS EQUIVALENT IN MILK CONTAINERS FOLLOWING A SINGLE ORAL ADMINISTRATION OF 200 mCi OF TRITIATED WATER

Time	Serum				Pancreas				Fat			
d	[illegible]-2b	B-132	B-135	[illegible]-175	B-26	B-132	B-135	J-175	B-26	B-132	B-135	J-175
0.3	381	344	—	—	73	112	243	98.8	24.8	—	17.9	11.9
1	354	—	322	—	159	96.4	139	135	—	118	83.8	131
1.4	361	403	380	425	145	—	—	287	—	—	—	224
2	326	284	235	358	123	151	154	177	78.9	149	—	72.1
2.4	289	298	206	315	114	112				73.9	76.2	
3	242	242	218	314	95.1	87*	129	119	72.8	91.5	75.4	68.5
4	240	257	237	318	69.8			102	67.6	65.7	56.3	60.5
5	178	244	201	249	58.6		54.5		75.5			51.1
6	91.6	249	147	180	44.1		47.8		57.7	53.1	42.8	36.9
7	72.7	153	124	145	35.3			96.5	26.5	36.8	33.5	37.2
9	39.8	95.8	75.8	—	25.2	43.8			34.7	28.7	22.6	23.8
11	22.8	54.8	43.4	26.7	4.38		18.37	25.8	6.86	19.6	20.2	
14	6.98	27.8	22.5	20.7	7.89	16.1	6.79		4.32	12.8	9.36	8.96
16	2.97	17.2	16.8	8.88	5.27	12.1	6.29		2.42	6.79	5.86	4.42
21	0.985	4.45	7.29	2.46	2.71	4.42	3.52	3.26	1.88	2.58	2.48	2.04
24	0.775	3.04	3.85	0.778	0.954	2.58	4.85	2.52	0.479	1.62	0.955	0.943
28	0.317	1.27	0.752	—	1.04	0.727	1.43	2.55	0.336		0.518	0.598
31	0.248	P. 720	0.458	0.388	1.29	0.884		1.49				0.345
37	—	—	—	—	0.407	1.34	1.32	1.85	0.151	0.447	0.262	0.479
45	0.027	0.041	0.053	0.042	0.425	1.18	0.778	0.915	0.095	0.179	0.241	0.159
52	0.020	0.019	0.036	0.038	0.584	0.686	0.589	1.42		0.060	0.244	0.254
59	0.014	0.016	0.023	0.029	0.678	0.624	0.453	2.26	0.141	0.089	0.052	0.055
65	0.047	0.019	0.040	0.025	0.243	1.28	0.481	0.427	0.043	0.173	0.244	0.172
72	0.015	0.021	0.026	0.013	2.49	0.644	0.452	1.85	0.018	0.054	0.051	0.134
79	0.011	0.021	0.016	0.015	0.154	1.48	0.684	1.38	0.032	0.052	0.034	0.143
86	0.010	0.006	0.023	0.009	0.119		0.581	0.584	0.018	0.027	0.015	0.045
93	0.010	0.014	0.010	0.009	0.101	0.481	0.114	0.244	0.024	0.258	0.019	0.271
100	0.004	0.003	0.014	0.007	0.043	0.253	0.057	0.054	0.047	0.384	0.014	0.111
107	0.003	0.007	0.019	0.003	0.072	0.444	0.069	0.242	0.042	0.421	0.149	0.0963
114	0.004	0.043	0.005	0.005	0.060		0.172	0.074	0.041		0.038	0.130
120	0.005	—	0.005	0.010	0.048			0.114	0.017		0.025	0.0197

TABLE 2. [illegible] CONCENTRATION IN μCi/L OF WATER OR ITS EQUIVALENT IN MILK COMPONENTS FOLLOWING A SINGLE ORAL ADMINISTRATION OF 200 mCi OF TRITIATED WATER. (Continued)

Time (h)	Serum				Permeate			Fat				
	M-26	M-132	M-135	JMM-175	M-26	M-132	M-135	J-175	M-26	M-132	M-135	J-175
120	0.0052		0.0088		0.0041				0.0011		0.0021	
125	0.0026		0.0054		0.0057		0.0101	0.0142	0.0028		0.0027	0.0176
127	0.0054		0.0053		0.0054		0.0101		0.0048		0.0026	0.0139
142	0.0017		0.0051		0.0047		0.0088	0.0096	0.0022		0.0026	0.0051
144	0.0011		0.0057		0.0041		0.0066	0.0071	0.0021		0.0021	0.0047
149	0.0018		0.0086		0.0033		0.0035	0.0036	0.0021		0.0026	0.0017
156	0.0010		0.0064		0.0017		0.0026	0.0032	0.0020		0.0024	
158	0.0052		0.0065		0.0013		0.0035	0.0017	0.0014		0.0013	0.0013
163	0.0035		0.0051		0.0026		0.0051	0.0026				
177	0.0013		0.0052		0.0018		0.0054		0.0015			0.0049
165	0.0021		0.0057		0.0029			0.0026			0.0017	0.0049
191	0.0013		0.0013		0.0024		0.0041	0.0027	0.0016		0.0065	0.0054
198	0.0035				0.0038		0.0017	0.0035	0.0021			0.0071
205	0.0054				0.0079		0.00297	0.0043	0.0024		0.0024	0.0029
215	0.0021				0.00298		0.00215	0.0017				
226	0.0021				0.00197		0.0029	0.0024			0.0024	0.0023
235							0.0025				0.0066	0.0054
240								0.0013				0.0035
247								0.0017				0.0057
254								0.0036				0.00xx
261												0.0065
268												0.0036
275												0.0029
282												0.0017

*M = Robinson
**J = Jansen

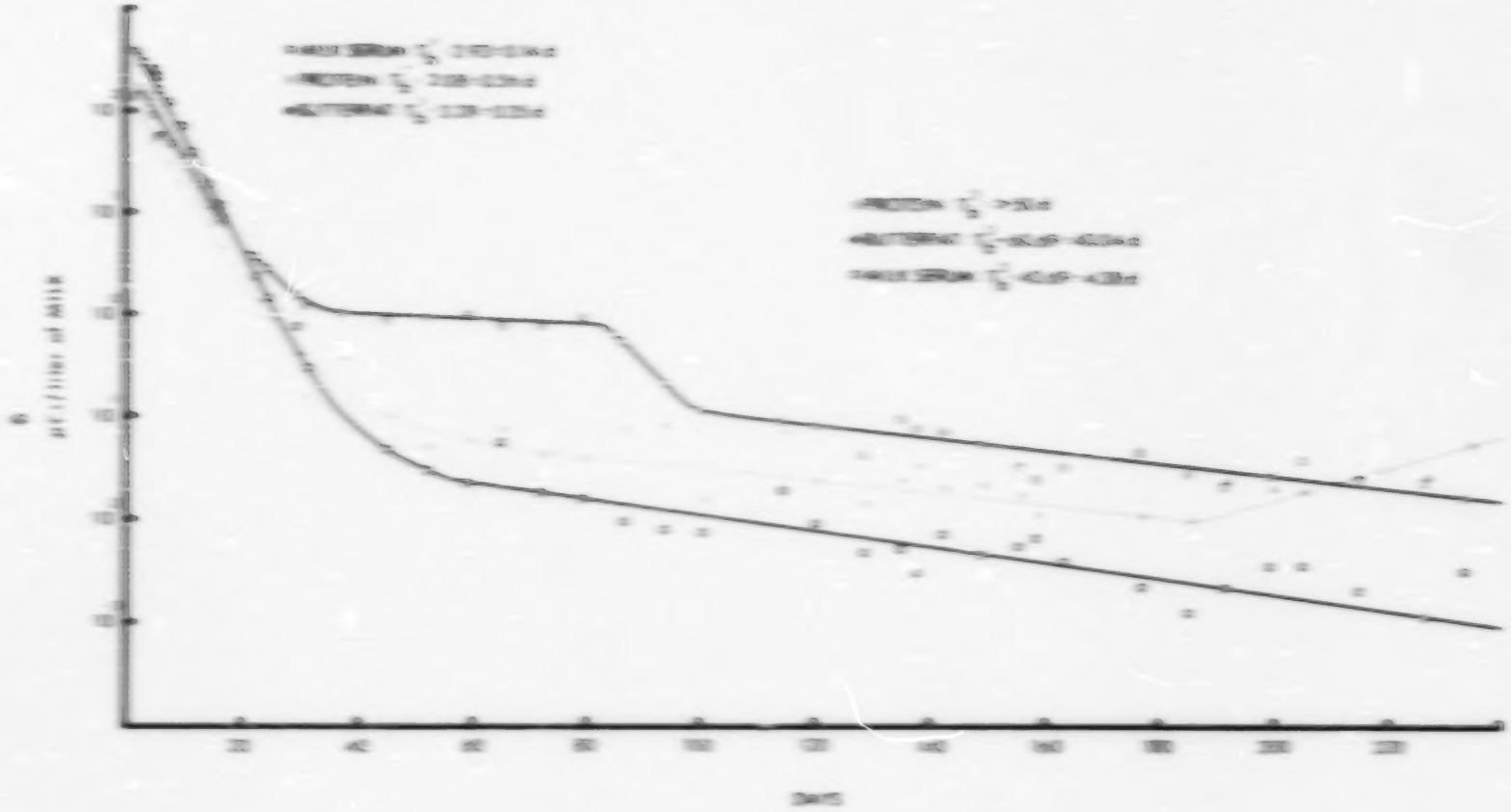

Figure 1. Average concentration of ceftiofur in milk components following a single oral administration of 200 mL of ceftiofur waste to four dairy cows.

TABLE 3: TRITIUM CONCENTRATION IN BLOOD SERUM OF DAIRY COWS FOLLOWING A SINGLE ORAL ADMINISTRATION OF 100 mCi OF TRITIATED WATER

Time h	H-21 µCi/l	H-90 µCi/l	H-171 µCi/l	I-175 µCi/l
1	124	250	136	112
2	242	118	326	374
3	259	303	410	406
4		330	426	414
5	250	298	413	417
6	238	295	430	411
7	243	305	428	440
8	248	294	427	441
9	335	291	417	410
10	229	279	404	421

TABLE 4: TRITIUM CONCENTRATION IN BLOOD SERUM OF DAIRY COWS FOLLOWING A SINGLE ORAL ADMINISTRATION OF 200 mCi OF TRITIATED WATER

Time d	H-110 µCi/l	H-132 µCi/l	H-135 µCi/l	I-175 µCi/l
1/24	171	240	150	272
2/24	313	320	197	425
3/24	308	355	306	505
4/24	354	403	334	437
5/24	378	391	340	507
6/24	429	418	394	495
7/24	369	406	359	488
8/24	356	397	359	491
9/24	373	384	362	457
10/24	247	365	340	449
11/24	331	377	362	507
12/24	343	401	375	492
2	38.2		58.1	87.6
16	2.98	16.2	9.93	8.70
24	0.629	2.87	2.74	1.52

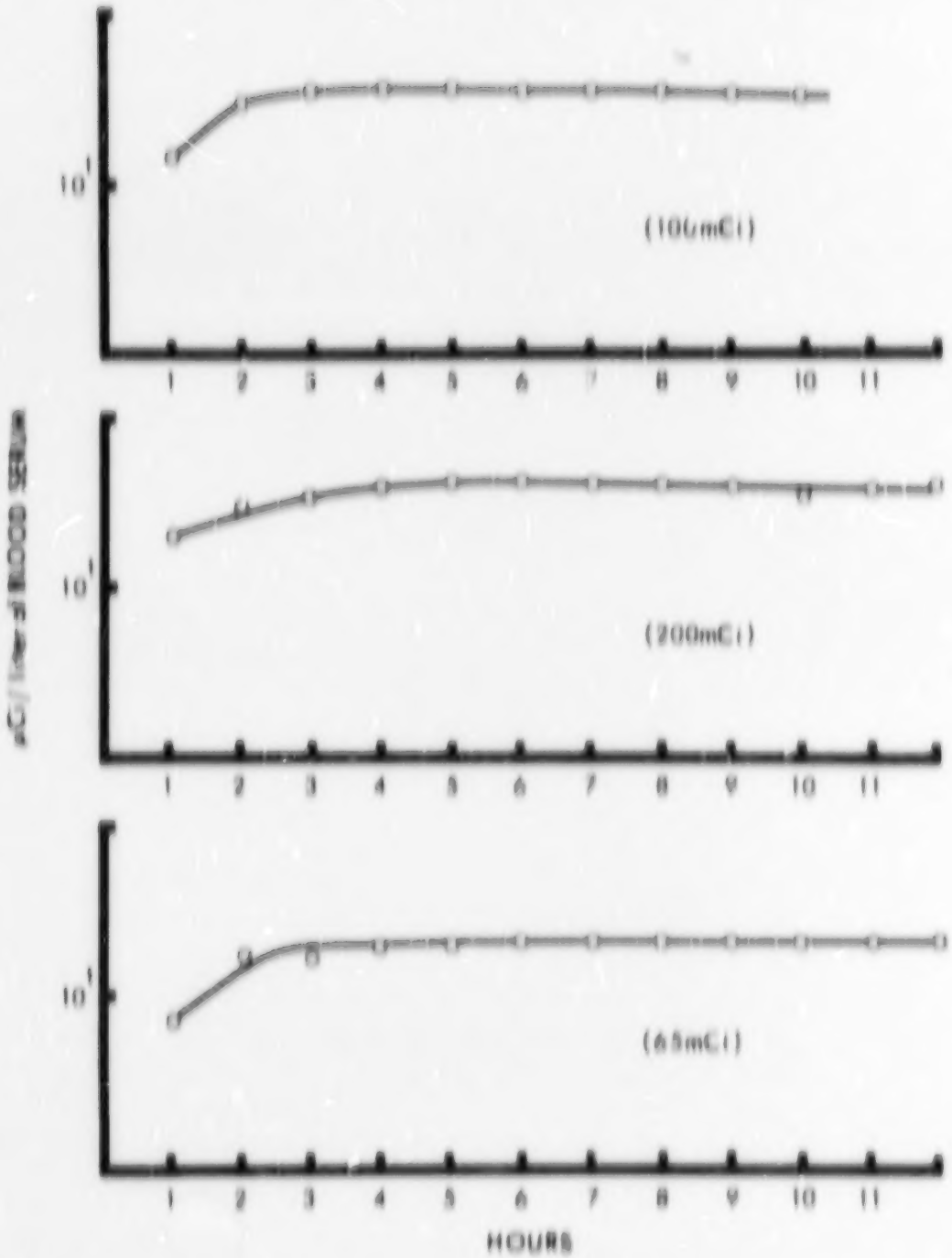

Figure 1. Average concentration of tritium in blood serum following a single oral administration of tritiated water to dairy cows and beef animals.

TABLE 5. TRITIUM CONCENTRATION IN BLOOD SERUM OF BEEF CATTLE FOLLOWING A SINGLE ORAL ADMINISTRATION OF 65 mCi OF TRITIATED WATER

Time d	Cow #662 µCi/l	Cow #669 µCi/l	Cow #675 µCi/l
1/24	---	---	72
2/24	137	118	194
3/24	163	126	165
4/24	174	191	184
5/24	181	219	194
6/24	215	223	202
7/24	218	256	216
8/24	201	239	206
9/24	184	219	207
10/24	206	255	212
11/24	202	240	214
12/24	210	265	215
1	208	221	216
2	135	122	174
8	140	144	105
16	28.3	37.1	---
23	11.4	12.1	11.2
28	4.45	5.51	4.84
30	1.92	2.09	3.21
65	0.071	0.096	---
72	0.064	0.063	0.065
79	0.056	0.076	0.075
86	0.044	0.039	0.047
93	0.028	0.078	0.031
100	0.065	0.067	0.040
114	0.029	0.023	0.030
121	0.038	0.030	0.020
128	0.028	0.021	0.018
135	0.023	0.018	0.025
139	0.015	0.018	0.017
142	---	0.018	0.022
144	0.018	0.017	0.045
149	0.020	0.023	0.084
156	0.009	0.011	0.012
158	0.015	0.021	0.010
161	0.010	0.009	0.009

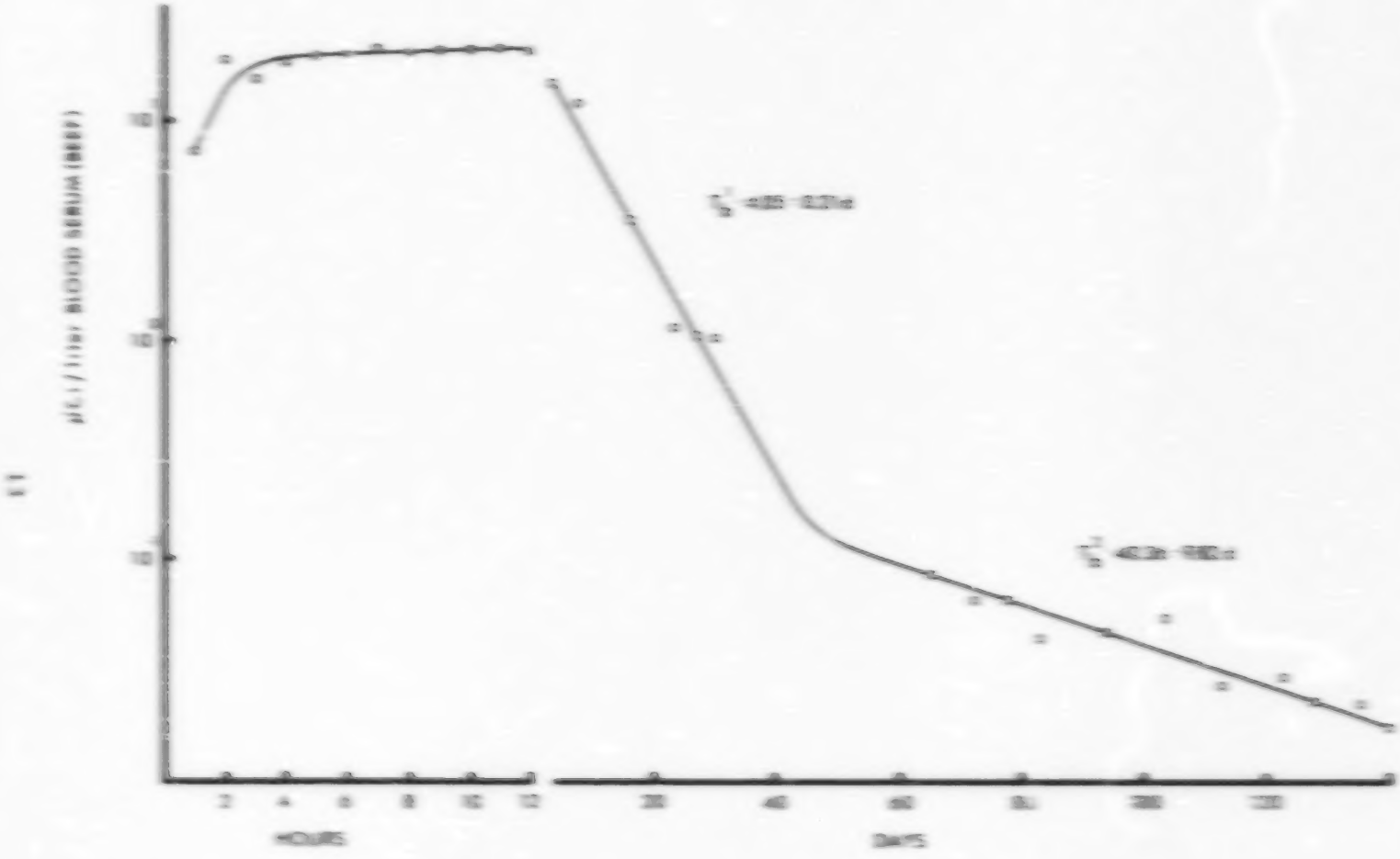

Figure 4. Average concentration of L-Ethal in blood serum following a single oral administration of 65 mCi of tritiated ethal to three beef cattle.

DISCUSSION

Results of this study show that the turnover rate of tritium in the body-water pool of lactating dairy cows is not appreciably different from nonlactating beef animals at 3 days and 4 days, respectively. The tritium turnover rate 60 days after ingestion showed even less difference, 44 days for lactating dairy cows and 40 days for beef animals. This result is similar to that noted by Black et al.,(1964) who found the average half-life for body water in lactating cows to be 3.54 ± 0.10 days and that in nonlactating cattle to be 3.40 ± 0.18 days over the course of a 15-day study following intravenously administered tritiated water to dairy cows and steers. The greater differences in T_b noted by Aschbacher et al.,(1965) 7 days for nonlactating and 5 days for lactating cows may have been due to the use of fat, nonlactating, nonpregnant cows, whereas the present study and Black's study utilized steers. There may be differences in the rate of tritium turnover between the two experimental groups due to the differences in formation and turnover of muscle by steers versus adipose tissue by nonlactating cows.

The difference in the rate of incorporation of tritium into proteins as compared to its incorporation into non-exchangeable portions to form stable carbon-tritium lipid bonds is indicated by the shorter T_b of 3.08 and about 50 days for protein in milk as compared to T_b of 3.28 and 97 days for butterfat. The concentration of tritium in fat was noted by Schirch and Mason (1963) while investigating concentrations in humans chronically exposed to tritium.

Moyer and duVigneaud (1972) found the appearance of tritium in protein was maximal after 1 day following intravenous administration to rats, but fell to about 20 percent of the peak level by day 5. Tritium incorporated into serum fatty acids became maximal during day 1 and remained relatively constant until the 12th day, whereas, the appearance of tritium in serum cholesterol increased over a period of 12 days. It would appear that the complexity of the chemical transformations of tritium (Smith and Taylor, 1969) does not allow a simple conclusion to be drawn concerning the fate of ingested tritium.

The relatively long half-time of >120 days for the transfer of tritium to whole milk indicates that milk may be the major contributor to the radiation dose of humans consuming tritium-contaminated dairy products. However, although milk solids constitute only 1/10 of the total volume of milk, the radiation dose resulting from consumption of butterfat and cottage cheese may exceed that from whole milk due to the longer biological half-lives and incorporation of tritium in fat, protein, and carbohydrates. It will be necessary to establish the turnover of tritiated milk solids in humans before it will be possible to accurately estimate the radiation dose resulting from the consumption of tritium-contaminated dairy products.

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